Sequence motifs, polar interactions and conformational changes in helical membrane proteins

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The α helices of transmembrane proteins interact to form higher order structures. These interactions are frequently mediated by packing motifs (such as GxxxG) and polar residues. Recent structural data have revealed that small sidechains are able to both stabilize helical membrane proteins and allow conformational changes in the structure. The strong interactions involving polar sidechains often contribute to protein misfolding or malfunction.

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Abbreviations

bR	bacteriorhodopsin
CFTR	cystic fibrosis conductance regulator
GpA	glycophorin A
MCP	major coat protein
MscL	mechanosensitive channel of large conductance
MscS	mechanosensitive channel of small conductance
PDGF βR	platelet-derived growth factor β receptor
TM	transmembrane

Introduction

Transmembrane (TM) proteins, which represent 20–30% of all open reading frames in sequenced genomes, form the functional basis of biological compartmentalization at the cellular level. However, to date, there are only 50 unique high-resolution structures of membrane proteins compared with thousands of their water-soluble counterparts (see http://blanco.biomol.uci.edu/Membrane_Proteins_xtal.html). Excepting β-barrel structures, TM helix interactions are a dominant theme. Several different approaches have been successful in furthering our understanding of how helices interact in TM proteins. Here, we examine key features that have become apparent: sequence motifs, polar interactions and conformational changes.

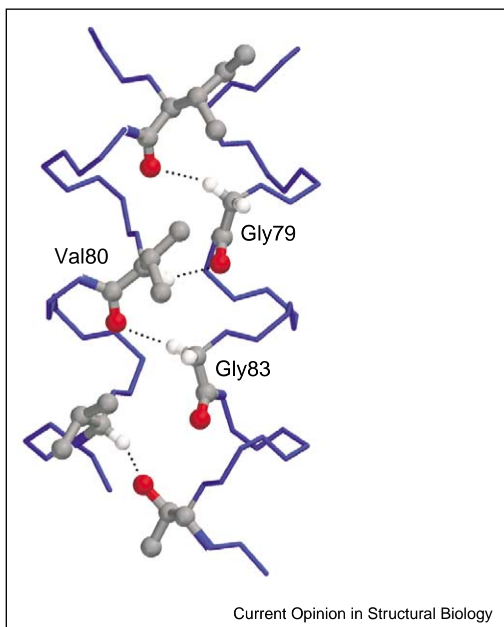
Membrane-spanning domains are composed mainly of α helices, whose associations within the cellular membrane are generally governed by electrostatic and van der Waals interactions. Other factors, such as ligand binding [1,2] and the folding of extramembranous loops [3,4], also contribute to the packing of TM helices. The folding of helical TM proteins may include two energetically distinct stages. The first stage sees the formation and insertion of α helices into the membrane and, in the second stage, these preformed helices interact within the cellular membrane. A further step, whereby additional structure re-enters the membrane, may be subsequent to the first two. The free energy change associated with helix–helix interaction in detergent micelles has recently been investigated [5].

Analysis of the helical packing within membranes has revealed that the most favored packing angle is left handed, at approximately 20° [6] (compared to right-handed at −35° for water-soluble proteins), although right-handed contacts do occur. Left-handed packing increases the interfacial area between the helices. The general packing of α helices in membrane proteins has been described by the ‘knobs-into-holes’ packing model first described for soluble coiled coils [7]. However, it has become clear that there is much more detail that needs to be considered if we are to develop a predictive understanding of helix interactions in membrane proteins. Of specific interest is the role of sequence motifs and polar residues. Here, we discuss their involvement in mediating helix–helix interactions in membrane proteins and the functional nature of such contacts.

Sequence motifs

Many concepts of how helices interact in membranes come from work on the TM domain of glycophorin A (GpA). GpA is a dimeric protein expressed on the surface of erythrocytes. Saturation mutagenesis studies have defined the dimeric interface of the TM domain as consisting of a right-handed seven-residue motif — LIxxGVxxGVxxT [8]. This interface was later confirmed by NMR studies [9]. More recently, general sequence motifs have emerged as important mediators of interactions between TM helices. Studies of the TM domain of the major coat protein (MCP) from bacteriophage also highlighted a GxxxG dimerization motif [10,11]. The GxxxG (GG_4) motif found in GpA and MCP has been shown to mediate strong helix association for several different TM domain sequences in a genetic screen of the inner membrane of *Escherichia coli* [12]. This same motif was highlighted in a survey of pairwise interactions as being the most statistically significant over-represented pair of amino acids in single-pass TM helices [13]. Other over-represented pairs include II_4, GA_4 and IG_1. The

Figure 1



The potential network of $C_{\alpha}H \cdots O$ hydrogen bonds at the GpA dimer interface. Apparent hydrogen bonds are represented by dots (···). The presence of the hydrogen-bonding network increases the stability of the dimer.

presence of β-branched residues in TM helices may facilitate helix–helix interactions by providing a conformationally restricted interface and has been highlighted in other studies [9,14–17]. Similar motifs emerge from conserved TM positions in a classification of membrane domain families in 26 genomes [18]. Additional analysis of the occurrence of glycine residues in membrane protein structures revealed that GG_4, along with other [small]xxx[small] motifs (where [small] represents alanine, glycine or serine), minimizes the steric hindrance of helix backbones. It has been suggested that this enables a $C_{\alpha}H \cdots O$ hydrogen bond to form across the helical backbone [19•]. The presence of such a hydrogen bond would enhance the stability of interactions between TM helices. Initial analysis of the structural database identified potential $C_{\alpha}H \cdots O$ hydrogen bonds in several membrane proteins, including GpA, Ca^{2+}-ATPase and the glycerol facilitator. This is illustrated for GpA in Figure 1. A more recent study has highlighted the prevalence of $C_{\alpha}H \cdots O$ hydrogen bonds in photosystem I [20]. This study indicates that such hydrogen bonds are important for guiding the specificity of packing interactions between TM helices.

Polar interactions

TM helices are known to contain few polar residues. In fact, with the exception of threonine and serine, which account for approximately 7% of the amino acids, the remaining polar amino acids constitute only 1–3%. However, as with water-soluble proteins, polar and charged amino acid residues are functionally important. Therefore, although the insertion of such residues into the membrane may be energetically unfavorable, there is a functional necessity to accommodate them, as well as a structural need. Studies have shown that several polar residues, namely glutamine, glutamic acid, aspartic acid and asparagine, are able to promote strong homo-oligomerization in a polyleucine or GCN4 leucine zipper sequence context, whereas the more frequently occurring serine and threonine residues do not [21,22•,23•]. A recent high-resolution structure of the mechanosensitive channel of small conductance (MscS) has identified two arginine residues that are adjacent to each other [24]. Although there is no direct evidence, it would seem probable that these polar residues, found within the membrane-spanning region, interact with one another.

Genetic selection for interacting TM interfaces in the absence of glycine residues identified SxxSSxxT and SxxxSSxxT as motifs able to mediate strong helix interactions [25]. It was suggested that, although single serine or threonine residues are unable to promote helix association, motifs containing these residues can drive strong and specific association through a cooperative network of interhelical hydrogen bonds [17,26]. Halorhodopsin, Ca^{2+}-ATPase and cytochrome *c* oxidase have been given as examples of proteins exhibiting such hydrogen-bonding networks. Polar interactions within membrane proteins can also play a major role in the formation of non-native contacts. This can result in protein misfolding or the formation of interactions with other protein domains. The mutation of a nonpolar residue to a polar one is commonly associated with disease [27].

A recent survey of the cystic fibrosis conductance regulator (CFTR) revealed that nonpolar to polar or charged mutations in the TM region of the protein account for many of the mutations that cause cystic fibrosis [28•]. The effect of these mutations has been studied [28•,29]. It was shown that a mutation located on TM helix 4, V232D, enables the formation of a hydrogen bond with the proximal wild-type residue Q207 found on TM helix 3. This suggests that such a hydrogen bond could alter the normal assembly of CFTR TM helices and/or impede their movement in response to substrate transport.

In two known cases, a receptor can be activated by TM interaction with a foreign protein, resulting in a signal cascade and aberrant cell proliferation [30,31]. In one case, the bovine platelet-derived growth factor β receptor (PDGF βR) interacts with E5, a TM peptide from the bovine papilloma virus. E5 binds as a dimer to two PDGF βR molecules, promoting receptor dimerization, phosphorylation and eventually cell proliferation [30]. Potential sites of contact between the TM domains of the two proteins have been identified. Specifically, a lysine

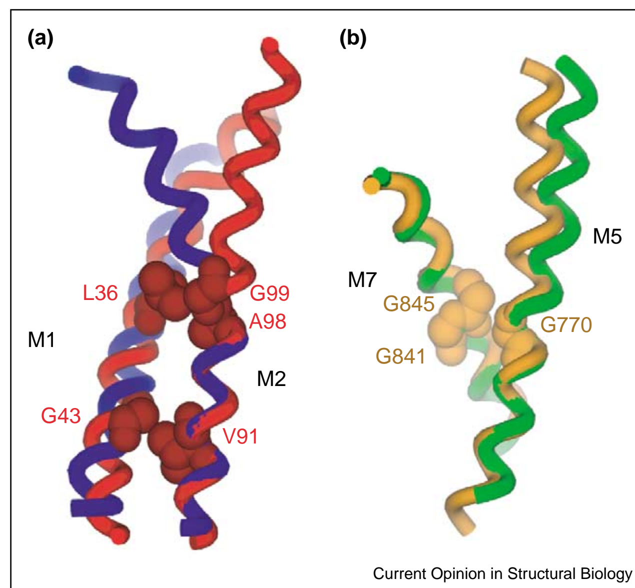
residue at the outer juxtamembrane position and a threonine residue buried within the TM domain of the receptor are required for stable interaction with the E5 protein [32]. Analogously positioned asparagine and glutamine residues on the E5 protein were also found to be necessary for complex formation [33]. Recent mutagenesis data have shown that the polar nature and relative position of the threonine residue are important for mediating this interaction [34,35]. It is of note that all of the amino acid residues identified in the interaction between the two TM domains are polar in nature.

Functional interactions

Helix–helix interactions are important for the formation and maintenance of three-dimensional structure in membrane proteins. In many cases, protein function requires that the conformation of the protein be flexible [36•]. A well-characterized example is bacteriorhodopsin (bR), the light-driven proton pump from *Halobacterium salinarium*. When bR is excited by a photon, the retinal chromophore isomerizes, promoting a cycle of photoreactions during which a proton is transferred to the extracellular environment. Several different structures of bR at various stages of the photocycle have been solved ([37–39] and references therein). These data have identified several small conformational changes that the protein undergoes during proton translocation.

In some cases, however, larger conformational changes need to be accommodated. One example of this is the potassium channel. The crystal structure of the closed state of KcsA, the potassium channel from *Streptomyces lividans*, revealed that the channel is composed of four membrane-spanning subunits, which form the exterior of a selective pore [40]. The TM helices of KcsA (M1, the outer helix, and M2, the inner helix) pack as coiled coils [41] with several points of contact. One such interaction — between L36 and G99 and A98, and also G43 and V91 — allows maximum contact for van der Waals interactions. It is of note that mutations G49W and G99W are among those that result in the disruption of the KcsA tetramer [42], suggesting that the tight packing of the two helices is essential for the formation of subsequent quaternary structure. A subsequent structure of a Ca^{2+}-gated potassium channel in the open state has been determined to a resolution of 3.3 Å [43]. This protein, the MthK channel from *Methanobacterium thermoautotrophicum*, is considered to be homologous to KcsA. The structure shows that the residue equivalent to G99, located on the inner helix in the KcsA structure, is important for the opening of the channel. It is at this point that the helices bend, with the glycine residue acting as the hinge point from which the bundle splays out, opening to a diameter of approximately 12 Å. This is compared with a pore diameter of 4 Å in the closed state [44••]. Consequently, the area of contact between the two helices is significantly reduced in the open conformation. Movement of the helices causes disruption of the van der Waals interactions, while maintaining the packing of the L39 'knob' of the outer helix in the G99–A98 'hole'. This interaction may be important for conserving the structural integrity of the selectivity filter between the open and closed states of these channels. The helical movement is shown in Figure 2a. Thus, the small sidechains may stabilize the closed state, preventing spontaneous opening and providing the flexibility needed for the conformational change.

Figure 2



The movement of TM helices resulting from the transition between the closed and open states of the potassium channel and Ca^{2+}-ATPase. **(a)** Helices M1 and M2 of the open (blue) and closed (red) states of the potassium channel. The interfacial positions G43 and V91, and L36, G99 and A98 in the closed state are shown in space-filling representation. In the closed state, the inner helices (M2) are straight, with a pore size of 4 Å compared to 12 Å in the open state. **(b)** Helices M5 and M7 of the open (yellow) and closed (green) states of the Ca^{2+}-ATPase. The packing interface, comprising a GG_4 motif on M7 (G841, G845) packed against a single glycine on M5 (G770), is shown in the open state.

A similar example is the Ca^{2+}-ATPase from skeletal muscle sarcoplasmic reticulum. A 2.6 Å crystal structure of the protein was reported in its Ca^{2+}-bound form [45] and, more recently, in a Ca^{2+}-free state [46]. The structure shows the Ca^{2+}-bound protein to be composed of three well-separated cytoplasmic domains and ten TM helices (M1–M10), although relatively few interhelical interactions are observed in the crystal structure. However, one such helix–helix interaction occurs between M5 and M7, and is mediated by the GG_4 motif located on M7 [19•]. Upon Ca^{2+} release, there is a major rearrangement of the cytoplasmic domains accompanied by movement of six (M1–M6) of the ten TM helices. It is significant that the bottom half of M5 (below E771) remains tightly

packed against M7, but the top (cytoplasmic) half of the helix tilts with G770 as the pivot point. This movement is illustrated in Figure 2b. As seen with the potassium channel, this movement disrupts the van der Waals contact of the GG_4 motif. Again, both stability and conformational change aspects of the small-sidechain residues are evident.

The structure of MscS, suggested to reflect the open state of the channel, has been determined to 3.9 Å [24]. The protein has been identified as a stretch-activated channel found in the inner membrane of *E. coli*. The channel is thought to undergo significant conformational rearrangements between the closed and open states, as proposed for the prokaryotic mechanosensitive channel of large conductance (MscL) [47••]. The TM domains of both MscS and MscL exhibit conserved [small]xxx[small] motifs at the interface of the pore-forming helices. Residues at this interface are required to accommodate the changes in packing between the helices in the different conformational states. It has been suggested that small amino acids located at the interface between the pore helices, specifically glycine and alanine, may facilitate interconversion between conductance states [48]. A recurring theme of small amino acid residues conferring conformational flexibility is observed.

Conclusions

Given the structure of the translocon [49•], it is most likely that helices are inserted into the bilayer one or two at a time. They must then interact to form higher order structures. The interactions involve both packing and polar interactions, including hydrogen bonds of different kinds. To facilitate packing and allow $C_{\alpha}H \cdots O$ hydrogen bonding, glycine is frequently used and, to a lesser extent, serine and alanine. These small sidechains are frequently found in motifs, such as GxxxG. Examination of the recent cases in which functional states have been documented reveals that the small sidechains can have dual roles — they both stabilize structure and allow changes in that structure.

Because of their role in stability, mutations that add polar groups to membrane domains can be a source of aberrant helix interactions. This in turn can contribute to protein misfolding or loss of function, as seen, for example, in cystic fibrosis.

The emerging insights regarding the chemistry of helix interactions and conformational changes should result in increasingly enabling views of membrane protein folding, function and diseases.

Update

The crystal structure of the potassium channel KirBac1.1 in the closed state has been determined to 3.65 Å [50]. The authors propose a model that implicates a glycine residue on the inner helix as being the point at which the helix bends. This is consistent with the ideas presented in this review, that glycine residues provide opportunities for helix packing while conferring structural flexibility.

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